Epidemiologie en preventie van nosocomiale infecties op de neonatale intensive care unit

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General Discussion and Summary

Nosocomial infections, also called hospital-acquired infections, arise as a consequence of admission to the hospital. In the hospital, the neonatal intensive care unit (NICU) is well known for its high incidence of nosocomial infections. This is caused by several factors. First, the patient population is highly susceptible to infection, due to a suboptimally functioning immune system. This applies to first-line defence mechanisms, such as the skin (thin and fragile) and the airways (reduced coughing reflex), as well as second-line defences, for example the cellular immune system. Many invasive procedures and devices undermine the first-line defences even further, for example intubation for mechanical ventilation and intravascular catheters for administration of fluids and medicines. Secondly, neonates are easily colonized by hospital microbial flora that is more often resistant to various antimicrobial agents. This is, amongst others, because neonates are sterile at birth, and thereafter intensively cared for and treated, which involves frequent hand contacts with health care workers. Therefore, contact with hospital bacteria is frequent, and as a consequence these bacteria are easily spread with a chance that they become epidemic. The most effective preventive measure is a thorough disinfection of the hands before patient contact. An ongoing effort is necessary to maintain a high level of adherence to hand disinfection protocols by health care workers; especially in periods of understaffing there is a tendency to omit disinfection of hands preceding to patient contact. Thirdly, in the NICU antimicrobial agents are frequently used for treatment of infection and suspected infection; this harbours the risk of selection of (multi) resistant bacteria, which makes treatment of infection more difficult.

In the second half of the preceding decade, the impression was that the occurrence of infections in the NICU of the VU University Medical Center, expressed as the number of infections per 1000 admission days, was high. We realized at that time, that little was known about the incidence of nosocomial infections in NICUs and on the specific risk factors for nosocomial infections in this patient population. There was no literature on Dutch NICUs and only few studies in the international literature. In addition, comparison with results of other studies was not possible because of the lack of standardized data from surveillance studies.

To confirm the impression that the local incidence of nosocomial infection in the NICU was high and to identify risk factors, we proposed to start a prospective study. The most important problem for investigation of the endemic level of infection in the NICU is the lack of standardized infection definitions for neonates. To our neonatologists, the CDC definitions for children < 1 year were not acceptable for neonates. In **chapter 2**, the major problems for designing such definitions in this patient category are described. We designed infection definitions based on existing CDC definitions intended for children < 1 year, but adjusted to the specific subtle symptomatology of infection in neonates and the usual diagnostic work-up of infection in the NICU. These adjusted definitions were then used for a surveillance period of 29 months. During this period, 742 neonates, who stayed in the NICU for more than 24 hours, were surveyed. Base-line risk factors, time-dependent risk factors (for example mechanical ventilation and various intravascular catheters), and the occurrence of infection were recorded prospectively on a daily basis.
Bloodstream infection and pneumonia were the most frequently occurring nosocomial infections. Through multivariate analysis, risk factors for infection were identified. The most important risk factor for all infections was low birth weight. Obviously, the administration of intravenous antibiotics was a protective factor. Other major risk factors were mechanical ventilation for pneumonia and the administration of parenteral feeding for bloodstream infection. It is known that the latter promotes the growth of coagulase negative staphylococci, the most frequent cause of blood stream infections in the NICU. Compared to American NICUs participating in the NISS, the use of devices, such as central venous catheters and mechanical ventilation, was high, which is a reflection of differences in treatment strategies between Dutch and American NICUs. This difference influences this comparison. The incidence of infection was high in our NICU, however, even after correction for the difference in

frequency of utilization of invasive devices. This can partly be explained by the differences in definitions for infection. Our definitions are more suitable for neonates than CDC definitions used for all children < 1 year of age. In a random sample of the infections that we recorded, it was shown that some of these would not been have been identified by CDC definitions.

In addition to the investigation of the endemic level of nosocomial infections in general, also the endemic level of infection and/or colonization with a specific bacterial species in the NICU can be investigated. An example is given in **chapter 3**. A neonate with a persistent infection with positive blood cultures with coagulase negative staphylococci over a period of three weeks despite treatment with vancomycin, was the reason to start an investigation. Eventually, this neonate died. With routine methods for measurement of antibiotic resistance in the medical microbiology laboratory, the CoNS-strain appeared susceptible to vancomycin. All other possible causes for treatment failure were excluded; all intravascular catheters were changed and levels of vancomycin measured in the blood were not different from levels measured in other neonates treated with vancomycin. The strain was identified as *Staphylococcus capitis*; it was heteroresistant to vancomycin, which means that the bacterial population contains a vancomycin resistant subpopulation. Because the great majority of the population is sensitive, the resistant subpopulation remains unnoticed with routine laboratory methods, but could have been the cause of therapy failure. This finding was the reason to investigate more than 200 CoNS strains from blood cultures from patients that been admitted to the NICU in the preceding three years, that were stored in the freezer. Forty-eight strains appeared heteroresistant to vancomycin and all these strains were identified as *S. capitis*. Genotyping of these strains with Amplified-Length Fragment Polymorphism (AFLP) showed that transmission of one *S. capitis* strain had occurred and that this strain had become endemic in the NICU. However, treatment of *S.capitis* infections with vancomycin in other neonates apparently did not cause major problems, since these infections remained unnoticed. It is difficult, however, to estimate retrospectively the true extent of the impact of infection with such heteroresistant strains in our NICU.

Shortly before and during the surveillance period described in this thesis, two epidemics with a specific causative microorganism that required specific control measures occurred. These outbreaks were thoroughly investigated, to detect sources of cross-infection. In addition, understanding the causes of an outbreak often leads to a better understanding of the risk factors to which the patient population is exposed and hence, to improved infection control.

In **chapter 4**, an outbreak caused by *Bacillus cereus* is described. This bacterium, which belongs to the normal flora of skin and mucous membranes and seldom causes infections in non-immunocompromised hosts, caused serious infections in three neonates within a short period; one of them died as a consequence of infection. Genotyping of the three causative *B. cereus* strains showed that they were clonally related and that transmission of one strain in the NICU had occurred. This observation prompted an extensive investigation into the cause and source of this epidemic. In the preceding years, colonization of the airways of neonates with *B. cereus* had been a frequent observation in sputum specimens from the NICU, but this had never been paid much attention to because this bacterial species was considered colonisation or contamination.
Bacillus species can survive in the environment as spores for long periods of time. A spore is a dormant structure for survival of the cell, in which DNA is stored in a resistant envelope and in which there is no cell metabolism or cell fission. Characteristic is the ability of these spores to survive various unfavourable circumstances that would be lethal to normal cells, such as dryness, very high temperatures, high or low acidity or radiation. Even chemicals, such as disinfectants are resisted by spores. Spores can survive for long periods, and regain viability under favourable circumstances. Therefore, also the hospital environment can become contaminated with spores of *Bacillus* species.
Initially, *B.* cereus was isolated from only 1 of 32 environmental specimens, but this environmental strain was different from the strain that caused the infections. In a prospective study of 22 neonates, it was found that colonization with *B. cereus* solely occurred in the respiratory tract. The genotype of these colonizing strains was identical to the epidemic strain. Subsequently, several characteristics and

potential risk factors of these 22 colonized neonates were compared with those of 22 non-colonized neonates. Mechanical ventilation with a Sensormedics machine appeared to be a significant risk factor for colonization. This led to a new environmental study for the presence of *B. cereus*, this time of various materials used preceding or during mechanical ventilation in the NICU. Several balloons that were used for manual ventilation appeared to be contaminated with *B. cereus* spores, identical to the epidemic strain. It appeared that after use, these balloons were only cleaned superficially, which is insufficient to kill spores.
After the identification of the balloons as a possible source of the epidemic strain of *B. cereus*, all ventilation balloons on the ward were autoclaved (heated at high temperatures with saturated steam, which kills spores effectively) and with this measure the source of colonization of neonates with *B. cereus* was eliminated and no new serious infections caused by this bacterium occurred anymore. Thereafter, regular sterilisation of these balloons was introduced as a routine infection control measure. Hence, this outbreak led to the recognition of a previously unidentified potential source of nosocomial infections in the NICU and to better infection control.

The second outbreak is described in **chapter 5.** It was caused by a *Klebsiella pneumoniae* strain that was resistant to gentamicin. Since, at that moment, the empiric antibiotic regimen for serious neonatal infection on the NICU was a combination of ampicillin and gentamicin, this was a serious problem, because *Klebsiella* species are also naturally resistant to ampicillin. The first isolate was cultured from a routine sputum specimen. Thereupon, a *K. pneumoniae* strain with an identical antibiogram was isolated from a blood culture drawn from a sick premature neonate, who died 6 days later. Possibly, the delayed administration of effective antibiotic therapy had contributed to the death of this neonate. Thus, it was likely that transmission of a multiresistant *K. pneumoniae* strain had occurred. The infection control team drew renewed attention to thorough disinfection of the hands by health care workers between patient contacts as this had been the most probable route of transmission. Despite this action, another 4 neonates became colonized in the following 6 weeks, most frequently in sputum but sometimes also in urine. These colonized neonates were cohorted as much as possible, which means that they were treated and cared in the same room and by the same nurses, separated from non-colonized neonates. Cultures from possible environmental sources were negative for gentamicin-resistant *K. pneumoniae*. Genotyping of 8 gentamicin-resistant *K. pneumoniae* strains revealed 7 identical strains, which was proof of transmission in the NICU.
Preventive measures appeared insufficient as five other neonates became colonized shortly after they had been admitted to the NICU. Two of them developed pneumonia with this bacterium. At this moment, there was a shortage of nursing staff and because of this the workload was high. Investigation of whether certain factors increased the risk of becoming colonized or infected was done by comparing colonized neonates with those that were not colonized (case-control study). Low birth weight appeared the only significant risk factor. These neonates are more intensively treated and cared compared to heavier neonates, which involves more hand contacts. Eventually it was decided to ban the use of gentamicin on the ward to remove the selective advantage for this gentamicin-resistant bacterium. Gentamicin was replaced by amikacin, a related aminoglycoside with nearly the same spectrum but to which resistance is more difficultly developed. In the succeeding weeks, this intervention was monitored by frequent surveillance cultures of all admitted neonates. The intervention proved successful, as only one more patient became colonized with gentamicin-resistant *K. pneumoniae*.
This second epidemic is an example of an outbreak that is not caused by a specific common source but by selection, and hence facilitated spread, of a single bacterial strain due to its antibiotic resistance.

In the genesis of infection, the protective activity of the immune system of the patient is of major importance. In the neonate, the innate immune system and specific immune system are still developing and function suboptimally. This is especially true for premature neonates. We investigated two aspects of this complex system: transferred maternal humoral immunity [exemplified by maternally acquired antibodies against the varicella-zoster virus (VZV)] and a member of the innate immune system, the Mannose Binding Lectin (MBL).

During pregnancy, neonates acquire protective antibodies against VZV. This virus causes chickenpox, which can be a very serious disease in neonates. VZV can be inadvertently be introduced into the NICU by persons who are in the incubation period of this infection. When this person is diagnosed with chickenpox, all neonates that have been in contact with this person are screened for the presence of maternally acquired VZV immunoglobulins. VZV-IgG immunoglobulins are administered to neonates with low or absent VZV immunoglobulins. This does not protect them from getting chickenpox, but it mitigates the severity of the disease.
At the time of study, it was thought that preterm neonates (born at a gestational age < 37 weeks), and especially very preterm neonates (born at a gestational age < 28 weeks) would have acquired insufficient amounts of maternal VZV immunoglobulins to be protected from chickenpox, and that the titer would decrease faster to non-protective levels compared to term neonates. In **chapter 6** we describe the investigation of blood specimens of 43 mothers and 211 neonates for the presence of VZV immunoglobulins. Furthermore, de VZV-IgG titer of 27 neonates was followed over time, by obtaining sequential specimens. We determined that the half-life of VZV immunoglobulins in preterm neonates was identical to that in older children and adults, but that significant variability occurs in the first weeks of life. In our study, the neonatal VZV-IgG titer was predominantly predicted by the maternal VZV-IgG titer and to a lesser extent by the gestational age. Actually, a significant proportion of neonates that were candidate for receiving VZIG, were VZV-IgG positive. In three cases the mother was VZV-IgG positive but her child tested negative. We were not able to explain these discrepancies.

Mannose Binding Lectin (MBL) is a major component of the aspecific immune system. This serum protein is produced by the liver and can bind to the surface of various microorganisms, and, by doing so, activate the complement system which kills the microorganism. In some individuals, a change (mutation) of the DNA, which is involved in the production of MBL, causes a lowered production of MBL or the production of dysfunctional MBL. If other components of the immune system also act sub optimally, a low MBL titer is a risk factor for infection. Two examples are children of 6-17 months of age in which maternal immunoglobulins have disappeared and which have not developed their own immunity, and older patients treated with chemotherapy. We hypothesized that a low-level MBL in neonates would increase the susceptibility to nosocomial infection, because of the suboptimal function of other parts of the immune system in this patient category. In **chapter 7** we describe a study with a subpopulation of 186 neonates from the surveillance study that is described in chapter 2. We investigated blood spots on Guthrie cards from these neonates for variance of the DNA region that encodes MBL. For this subpopulation as well, a low birth weight was the most important risk factor for infection. We found no relation between genetic variations in the MBL-gene and infection risk. This can partially be explained by the fact that MBL binds poorly to CoNS, which are the most frequent cause of infection in neonates in the NICU. Also, it was recently found that the MBL-genotype is not perfectly correlated to the circulating and functional MBL titer; the latter is also influenced by other factors. We concluded that variation in the MBL genotype is not a good predictor for the infection risk in premature neonates.

Epilogue

The Neonatal Intensive Care Unit combines patients with a suboptimal immunesystem, frequent use of invasive procedures, intensive treatment, and frequent use of antibiotics, which makes it the ideal site for nosocomial infections to develop.
We described specific infection definitions, which comprised the subtle, aspecific symptomatology of infection in neonates, with which we could study the epidemiology and risk factors of nosocomial infection, of which little was known when we started this PhD study. Birth weight proved to be the overwhelmingly most important base-line risk-factor for nosocomial infection. When studied in a multivariate manner, none of the other base-line factors, including MBL-genotype, appeared to play a major role as a risk factor in comparison to birth weight. Of the time-dependent variables, mechanical ventilation appeared an independent risk factor for pneumonia, and intravenous feeding an

independent factor for bloodstream infection. Cox-regression with time-dependent variables proved an appropriate method to study risk factors which can change during admission.
Duration of pregnancy determines birth weight, and need for mechanical ventilation and parenteral nutrition. Hence, every effort directed at the prevention of preterm delivery will indirectly lead to less nosocomial infections in the NICU.
The selective pressure from the frequently used antibiotics in the NICU, combined with the frequent hand contacts between health care workers and neonate, causes an high intrinsic risk of transmission of especially resistant microorganisms. Our halting of an outbreak of resistant *K. pneumoniae* illustrates how a change of antibiotic policy may be an excellent adjuvant to classical infection control measures.
In the NICU, new medical devices/procedure are frequently introduced, without a thorough evaluation of their concomitant risk of infection. The outbreak of a *Bacillus cereus* strain, that was traced to balloons for manual ventilation, is a clear example of the continuous threat posed by medical devices to the natural defences of the human body. In addition, this outbreak illustrated that in an immunocopromised population every single bacterial species must be considered as a potential nosocomial pathogen. Indeed, we concluded that *Bacillus cereus* can cause serious infections in neonates and that it is mandatory to "be serious about *B. cereus*".
The contribution of genotypic fingerprinting in investigation of the above mentioned endemic or epidemic bacterial strains appeared indispensable.
Finally, obviously, prematurity means a premature immune system. We found no relationship between the Mannose Binding Lectin genotype and the risk for nosocomial infection. This is probably caused by the weak correlation between MBL genotype and phenotype and possibly by the poor binding of MBL to coagulase-negative staphylococci, which are the most frequent cause of infection in neonates. In addition, the important role of birth weight as a baseline risk factor, may mask the effect of other factors. This means that much larger studies may be needed to uncover the role of other base-line risk factors than birth weight.
Surprisingly, in contrast to general opinion, gestational age, and hence birth weight, was not the most important predictor of the neonatal VZV-IgG titer. Maternal protection, in this case provided by high maternal VZV-IgG titers, is the best there is for every neonate.